Malaysian Applied Biology (2023) 52(4): 65-72
https://doi.org/10.55230/mabjournal.v52i4.d137

Research

Effects of Non-Allelic Interactions of *O2* and *SU2* Mutant Genes on Grain Biochemical Composition in Various Corn Inbreds

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ABSTRACT

The use of combinations of non-allelic mutant genes of the maize endosperm structure creates opportunities for improving the quality of corn grain in comparison not only with forms of the common type but also with monogenic endospermic mutants. In this study, the effect of a combination of mutant genes *O2* (Opaque-2) and *SU2* (Sugary-2) according to the biochemical composition of the grain was studied. For the research, a series of inbreds - carriers of a combination of mutant genes *O2SU2*, inbreds - carriers of monogenic mutations *O2* and *SU2*, as well as maize inbreds of the common type of two-year reproduction were used. In the experiments, the content of protein, starch, and oil and the main characteristics of their quality were studied. It was found that the inbred carriers of the *O2SU2* combination are superior to the inbred carriers of monogenic mutations *O2* and *SU2* in terms of complex biochemical characteristics. In comparison with mutants O2 they were distinguished by an increased content of protein (by 12.3% on average), amylose in starch (by 38.9% on average), starch digestibility (by 24.4% on average), oil content (by 18.4% on average) and oleate content in oil (by 29.9% on average). In comparison with the carriers of *SU2* mutation, they had a higher content of lysine and tryptophan in the total grain protein (on average, by 19.4% & 14.3%, respectively). The main characteristics of grain quality in carriers of a combination of mutant genes *O2SU2* were characterized by quantitative variability, which can modify the effect of non–allelic interaction of mutant genes *O2* and *SU2*. The obtained results indicate the effectiveness of using non-allelic interactions between the *O2* and *SU2* mutant genes to improve the quality of corn grain.

Key words: Biochemical effect, endospermic mutants, *Zea mays* L.

Article History
Accepted: 24 July 2023
First version online: 31 October 2023

Cite This Article:
Tymchuk, D.S., Sadovnychenko, I.O., Tymchuk, N.F. & Pasiuga, O.S.
Serhiiovych, T.D., Oleksandrovych, S.L., Fedorivna, T.N. & Serhiivna, P.O. 2023. Effects of non-allelic interactions of *O2* and *SU2* mutant genes on grain biochemical composition in various corn inbreds. Malaysian Applied Biology, 52(4): 65-72. https://doi.org/10.55230/mabjournal.v52i4.d137

INTRODUCTION

To date, about 20 mutant genes of the endosperm structure with a useful biochemical effect have been identified and mapped in corn (Coe & Shaeffer,2005). However, the most commonly used among them are mutant genes that improve the amino acid composition of protein and the carbohydrate composition of grain.

Among the mutations that improve the amino acid composition of the protein, the most commonly used is the mutation *O2* (Opaque-2), which is located on chromosome 7. The mutant *O2* gene causes depression in the formation of alcohol-soluble endosperm proteins, limited in the content of essential amino acids, primarily lysine and tryptophan. Therefore, the content of these amino acids in the total grain protein of *O2* mutation carriers increases significantly (Prasanna *et al*., 2001).

Mutations that improve the carbohydrate composition of grain include mutations that redistribute the fractional composition of starch and mutations that change the content of water-soluble fractions of carbohydrates (Motto *et al*.,2010).

The biochemical effect of endospermic mutations has a local character. Mutation *O2* does not cause significant changes in the carbohydrate composition of grain, and starch-modifying mutations – changes in the quality of protein (Tymchuk *et al*., 2004). Carriers of the *O2* mutation, in addition, have a soft floury consistency of the endosperm, which causes an increase in grain injury and its damage by fungal diseases (Gibbon *et al*.,2003). Therefore, there is a need for further genetic improvement of corn based on endosperm mutations. There are at least two ways to solve this problem.

The first of these is the use of the effect of modifier genes (Sofi *et al*., 2009). Using this method, it was possible to obtain high-lysine corn with a grain consistency approaching normal, but no significant improvement in the quality of carbohydrates was found in this type of corn (Cisse *et al*.,2013).

The second method is to use the effects of non-allelic interactions between mutant genes of the endosperm structure that control the amino acid composition of the protein and starch-modifying genes, in particular, genes *O2* and *SU2* (Vikal & Chawla,2014). In this case, an improvement in the structure of the endosperm of high-lysine corn is also achieved, however, carriers of a combination of *O2SU2* may also have an improved carbohydrate composition of the grain.

In addition, obtaining this combination is technically simpler than obtaining favorable combinations of the *O2* gene with modifier genes. This is because the mutant genes *O2* and *SU2* cause the formation of specific visually diagnosable grain phenotypes (Shannon *et al*., 2009).

The *SU2 (Sugary-2*) gene is located on chromosome 6 and causes a decrease in the activity of soluble starch synthase *SSII*, leading to an increase in the amylose content in starch up to 40% (White,2001; Zhang *et al*.,2004).

It was found that the carriers of this mutation have a dense horn-like structure of the endosperm and in comparison to the corn of the common type have improved grain quality and, in particular, increased starch attackability during amylolysis (Gerard *et al*.,2001). In terms of the content of essential amino acids in the protein, they are somewhat superior to corn of the common type but inferior to carriers of the *O2* mutation. Therefore, the use of non-allelic interactions of the mutant gene *SU2* with the *O2* gene, which has a good protein quality, is of practical interest (Kumar *et al*., 2019).

Studies of carriers of the corn gene combination *O2SU2* have shown that they also have a dense vitreous grain phenotype, but are characterized by a higher content of essential amino acids in the protein, comparable to carriers of the *O2* mutation (Vikal & Chawla, 2014).

However, it remains unclear what is the effect of non-allelic interactions between *O2* and *SU2* genes according to the starch and amylose content, starch digestibility as well as the oil content and fatty acid composition, and whether the carriers of the *O2SU2* combination are superior to the carriers of the *O2* and *SU2* mutations in this respect. In addition, there is a need to establish the range of variability of grain quality traits for various sources of the *O2SU2* combination, which makes it possible to determine the perspectives of enhancing these traits by using the effects of polygenic complexes.

Therefore, the effects of non-allelic interactions between the mutant *O2* and *SU2* genes in terms of the content and composition of the grain proteins, carbohydrates, and lipids cannot be considered fully established. The determination of these effects was the purpose of the present study.

MATERIALS AND METHODS

Materials

The object of research was three groups of corn inbreds: the carriers of monogenic endospermic mutations *O2* and *SU2*, as well as carriers of the gene combination *O2SU2.* Each group of material was represented by 10 unrelated inbreds. 10 unrelated inbreds of common type that did not carry any endospermic mutation were used as controls.

The research material was received from the National Center for Plant Genetic Resources of Ukraine.

Soil and climatic conditions of field experiments

The growing of corn inbreds was carried out at a breeding and seed-production station "NASKO", located in the Novo-Kakhovsky district of the Kherson Region (Steppe Zone of Ukraine). The geographical coordinates of the place of field experiments are 46.7545^o Northern latitude, 33.3486^o Eastern longitude. The height above sea level is 15 m (Nova Kakhovka info, 2021).

The soils at the experimental site were represented by Southern black earth with a humus content of 1.5%, soil acidity pH=6.7, a weighted average phosphorus content of 8.54 mg/100 g, and a potassium content of 21.5 mg/100 g.

The climate of the experimental zone is temperate continental. According to observations of the meteorological station in Novaya Kakhovka (Synoptic index 33869), the average air temperature during the growing season of corn over the past 50 years is 18.5 °C, and the average annual precipitation is 239 mm (Nova Kakhovka, Ukraine Weather History, 2021).

The temperature conditions of the growing season were favorable for growing corn, but during this period, a deficit of atmospheric moisture was observed on the experimental plot.

Methods of Field Experiments

Field experiments were carried out according to the generally accepted method (Dospekhov, 2011), taking into account the zonal features of corn cultivation. To compensate for the lack of atmospheric moisture, the experiments were carried out under artificial irrigation conditions with a watering rate of 4000 m^3/ha, and soil fertility was ensured by applying mineral fertilizers at a dose of $N_{60}P_{60}K_{45}$.

Corn inbreds were sown manually on twenty nest plots with an accounting area of 4,9 m^2 in a double repetition for each experimental variant of the experiment. The placement of plots in the blocks of carriers of each endospermic mutation was carried out by the method of randomized repetitions. The sowing scheme was dotted with a row spacing width of 70 cm and a distance between plants in a row of 35 cm. To obtain the seeds within each plot, controlled re-pollination of 3-4 female inflorescences with pollen collected from the panicles of 5-6 other plants of the same plot was carried out. Identification of the allelic state of the genes of the endosperm structure was carried out by the phenotype of seeds. Two-year harvest inbreds were used for the experiments.

Methods of biochemical analysis

The protein content in the grain was determined by the Kjeldahl semi-micromethod, the lysine in the protein - by the colorimetric method with ninhydrin, and tryptophan in the protein - by the colorimetric method with para-dimethylaminobenzaldehyde (Yermakov,1987).

The starch content in the grain was determined by the Evers polarimetric method (Yermakov,1987), and the amylose content in starch - by the B.O.Juliano colorimetric method (Juliano,1971). The attackability of starch was determined by the rate of its decomposition in 1 and 4 h under the action of a standardized preparation of pancreatin with an α-amylase concentration of 32 mg /mL and an activity of 1370 U /mg (Knutson *et al*., 1982).

The oil content in the grain was analyzed by the gravimetric method of S.V. Rushkovsky (Yermakov, 1987) and the fatty acid composition of the oil - by the modified Peisker gas chromatographic method (Tymchuk *et al*., 2021).

Methods of statistical processing of experimental results

The obtained results were statistically processed by the method of variance analysis (Vukolov, 2008). In the course of statistical processing, the averages for each variant of the experiment and the errors of the averages (s_x) were calculated. The comparison of the averages for the experimental variants was carried out using the least significant difference (LSD), which was calculated for 5% of the significance level.

RESULTS

Content of protein and essential amino acids

Carriers of the *O2SU2* gene combination in terms of protein content in grain exceeded the inbreds of common type and carriers of *O2* mutation (on average by 10.2% & 12.3% respectively) and deviated towards the carriers of the *SU2* mutation (Table 1).

Table 1. The contents of protein and main essential amino acids in the grain of corn inbreds – carriers of *O2SU2* gene combination in comparison with the inbreds of other types, two–year test results

Types of inbreds	Protein content in the grain, % *			Lysine content in the protein, % *			Tryptophan content in the grain, % *		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Common	9.9	11.8	10.8 ± 0.2	2.4	2.8	2.6 ± 0.1	0.4	0.6	0.5 ± 0.1
Mutants *O2*	9.8	11.4	10.6 ± 0.2	3.8	4.5	4.2 ± 0.1	0.8	0.9	0.9 ± 0.1
Mutants *SU2*	11.4	13.3	12.1 ± 0.2	2.8	3.3	3.1 ± 0.1	0.6	0.7	0.7 ± 0.1
Mutants *O2SU2*	10.7	13.2	11.9 ± 0.2	3.4	3.9	3.7 ± 0.2	0.7	0.9	0.8 ± 0.1
LSD $_{0.05}$	0.6		0.5	0.3		0.2	0.1		0.1

*-Characteristic values: Min.-Minimal; Max.-Maximal; Mean-Average

In terms of the content of lysine and tryptophan in the protein the inbreds - carriers of the combination of mutant genes *O2SU2* significantly exceeded the inbreds of common corn and the inbreds - carriers of the *SU2* mutation and deviated towards carriers of the mutation *O2*.

Starch and amylose content in starch

Carriers of the *O2SU2* combination were inferior in starch content to the inbreds of common corn and the carriers of the *O2* mutation, but the average content of amylose exceeded the inbreds of both these groups. The mutants *O2SU2* deviated towards the carriers of the *SU2* mutation, surpassing them in starch content and inferior to them in amylose content (Table 2).

Table 2. The contents of starch and amylose in the grain of corn inbreds – carriers of *O2SU2* gene combination in comparison with the inbreds of other types, two–year test results

Types of inbreds	Starch content in the grain, % *			Amylose content in the starch, % *		
	Min.	Max.	Mean	Min.	Max.	Mean
Common	62.7	65.5	64.1 ± 0.3	25.5	27.1	26.3 ± 0.4
Mutants *O2*	63.2	64.5	63.8 ± 0.4	25.2	26.2	25.7 ± 0.3
Mutants *SU2*	56.3	58.4	57.4 ± 0.3	35.5	38.2	37.2 ± 0.3
Mutants *O2SU2*	59.8	62.8	61.5 ± 0.3	34.8	36.4	35.7 ± 0.4
$LSD_{0.05}$	1.0		0.8	1.0		0.6

*-Characteristic values: Min.-Minimal; Max.-Maximal; Mean-Average

Starch attackability

An increase in the amylose content in the starch of carriers of the *SU2* mutation and the *O2SU2* gene combination was accompanied by an increase in the attackability of their starches during pancreatic hydrolysis in comparison with common corn and carriers of the *O2* mutation. According to this feature, carriers of the *O2SU2* gene combination deviated towards carriers of the *SU2* mutation (Table 3).

Table 3. Attackabilities of starch during pancreatic hydrolysis in corn inbreds - carriers of *O2SU2* gene combination in comparison with the inbreds of other types, two–year test results

Types of inbreds	Starch attackability, % of hydrolysed starch *					
	Duration of hydrolysis 1 h			Duration of hydrolysis 4 h		
	Min.	Max.	Mean	Min.	Max.	Mean
Common	15.8	19.2	17.1 ± 1.1	57.8	61.7	59.9 ± 0.8
Mutants *O2*	17.4	19.6	18.4 ± 1.0	61.2	62.2	61.6 ± 0.8
Mutants *SU2*	27.8	30.4	28.9 ± 0.7	72.1	77.1	74.5 ± 0.8
Mutants *O2SU2*	29.4	33.7	31.3 ± 0.9	73.2	80.8	76.6 ± 0.9
$LSD_{0.05}$	2.6		3.7	2.6		3.7

*-Characteristic values: Min.-Minimal; Max.-Maximal; Mean-Average

The content and fatty acid composition of the oil

Carriers of the *O2SU2* gene combination had the highest grain oil content according to the experience, surpassing in this respect not only common corn but also the carriers of both monogenic mutations included in this gene combination (Table 4).

Table 4. The contents of oil in grain and glycerides of saturated fatty acids in the oil of corn inbreds - carriers of *O2SU2* gene combination in comparison with the inbreds of other types, two–year test results

Types of inbreds	Oil content in the grain, % *			Palmitate content in the oil, % *			Stearate content in the oil, % *		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Common	4.2	4.9	4.6 ± 0.2	10.5	13.9	12.3 ± 0.4	1.6	2.2	1.9 ± 0.1
Mutants *O2*	4.6	5.3	4.9 ± 0.2	10.2	13.9	11.6 ± 0.4	1.5	2.2	1.8 ± 0.1
Mutants *SU2*	4.7	5.8	5.2 ± 0.2	11.5	14.4	12.9 ± 0.6	1.7	2.6	2.1 ± 0.1
Mutants *O2SU2*	5.6	6.2	5.8 ± 0.3	12.5	14.9	13.4 ± 0.4	2.2	2.8	2.4 ± 0.1
$LSD_{0.05}$	0.7		0.3	1.3		0,9	0.3		0.2

*-Characteristic values: Min.-Minimal; Max.-Maximal; Mean-Average

The fatty acid composition of the oils in all the analyzed corn lines was represented by 10 individual components, but 2 minor components could not be identified, and the total content of 3 more components – palmitoleic, arachidic, and behenic acids accounted for only up to 3% of the total of all components of the fatty acid composition of the oil and these components did not play a significant role in ensuring the quality of the oil.

The highest content of saturated fatty acid glycerides was characteristic of carriers of the *O2SU2* gene combination, which, in terms of palmitate content, surpassed not only the common corn, but also the carriers of the *O2* mutation, and in terms of stearate content, also surpassed carriers of the mutation *SU2*.

The carriers of the *SU2* mutation and the *O2SU2* gene combination exceeded both common corn and carriers of the *O2* mutation in oleate content and were inferior to them in linoleate content. According to the content of these components of the fatty acid composition of the oil, the carriers of the

O2SU2 gene combination deviated towards the carriers of the *SU2* mutation (Table 5).

Table 5. The contents of unsaturated fatty acid glycerides in the oil of corn inbreds -carriers of *O2SU2* gene combination in comparison with the inbreds of other types, two–year test results

Types of inbreds	Oleate content in the oil, % *			Limoleate content in the oil, % *			Linolenate content in the oil, % *		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Common	23.3	28.8	25.3 ± 1.2	52.9	59.9	57.9 ± 1.4	1.1	1.6	1.3 ± 0.1
Mutants *O2*	24.1	29.3	26.1 ± 1.0	52.9	60.2	56.7 ± 1.2	1.2	1.7	1.4 ± 0.1
Mutants *SU2*	33.1	38.9	35.9 ± 0.8	43.8	50.3	47.0 ± 1.3	0.9	1.5	1.1 ± 0.1
Mutants *O2SU2*	31.9	36.6	33.9 ± 0.7	44.0	51.4	48.2 ± 1.2	0.6	1.3	0.9 ± 0.1
LSD $_{0.05}$	2.7		1.6	3.6		2.1	0.2		0.2

*-Characteristic values: Min.-Minimal; Max.-Maximal; Mean-Average

At the same time, among the carriers of the *SU2* mutation and the *O2SU2* gene combination, there were, although quite rarely, inbreds with an oleate content of about 27 - 28% and linoleate of 54 - 55%.

In the experiments, there was a tendency to a decrease in the content of linolenic acid glycerides in carriers of the *SU2* mutation and the *O2SU2* gene combination in comparison with common corn and the carriers of the *O2* mutation. However, the content of this component of the fatty acid composition of the oil was quite low and the use of its variability hardly makes practical sense.

The results obtained in the experiments showed that maize inbreds with the identical allelic state of the genes of the endosperm structure significantly differ among themselves in the levels of the main traits of grain quality and among the inbreds - carriers the *O2* and *SU2* mutations, as well as the *O2SU2* gene combination, it was possible to isolate inbreds with the most favorable biochemical composition of grain.

DISCUSSION

The results obtained confirmed that both partners of the *O2SU2* gene combination, mutant genes *O2* and *SU2,* are not only unlinked but also cause different biochemical effects. Therefore, if two independent mutant genes of this type exist in one genotype, three possible manifestations of their phenotypic effects should be expected.

The first of them consists of the simultaneous manifestation of traits controlled by each gene. The second manifestation of phenotypic effects in carriers of gene combinations is the deviation of a trait towards carriers of one mutation with a weak influence of the other. Finally, the third type of phenotypic effect consists of a synergistic increase or decrease in the trait in comparison with carriers of both monogenic mutations. The results obtained in our experiments showed that carriers of the *O2SU2* gene combination exhibited all three of the above types of phenotypic effects on the traits of corn grain quality.

The results obtained also confirmed the increased content of essential amino acids in the grain of carriers of the *O2SU2* gene combination in comparison with the usual corn and carriers of the *SU2* mutation. At the same time, the results of our studies show that carriers of the *O2SU2* gene combination have a higher protein content in the grain in comparison to common corn and carriers of the *O2* mutation. Although the increase in this trait may be the result of the effect of polygenic complexes (Cook *et al*., 2012), we believe that in our experiments, the main reason for the increase in the protein content in the carriers of the *O2SU2* combination was a decrease in the starch content due to the action of the *SU2* gene.

The effect of this gene, most likely, caused an increase in the amylose content in starch in carriers of the *O2SU2* combination in comparison with common corn and carriers of the *O2* mutation. In any case, such changes in the carbohydrate complex of the corn grain are characteristic of carriers of the *SU2* mutation (Campbell *et al*., 1994).

An interesting distinctive feature of the starches of carriers of the *O2SU2* gene combination was the high attackability of starches during pancreatic hydrolysis, significantly superior to the attackability of common corn starches and the carriers of the *O2* mutation. There is reason to believe that this feature is due to the biochemical effect of the mutant *SU2* gene. It has been previously shown that carriers of this mutation have a higher attackability of starch compared to common corn (Perera *et al*., 2001).

The increased content of amylose in the starch and the high attackability of starches create interesting opportunities for the practical use of carriers of the *SU2* mutation and the *O2SU2* gene combination, which are still very limited. However, it is known, that *SU2* mutant starches have technological properties that differ from usual corn starches (Tziotis *et al*., 2005).

It has been established that the oil content in corn grain is controlled mainly by polygenic complexes (Yang *et al*., 2012). However, this does not exclude the possibility of affecting this trait by monogenic endospermic mutations and their combinations. The most probable reason for the increase in oil content in the carriers of the *O2SU2* gene combination is a decrease in the mass of the endosperm and an increase in the proportion of the embryo, caused by a partial depression of starch formation.

The main feature of the carriers of the endosperm *SU2* mutation in corn and the combination of the *O2SU2* genes about the fatty acid composition of the oil was a decrease in the content of linoleate and an increase in the content of oleate. However, the results of studies carried out to date do not give grounds to link these changes in the fatty acid composition of the oil with the direct effect of endosperm mutations. It is known that the mechanism for increasing the oleate content consists in inhibiting the synthesis of linoleic acid from oleic acid, catalyzed by desaturase *FAD-2* (Dar *et al*., 2017). Currently, some oleate-coding loci have been identified in maize, one of which is located on chromosome 6 (Wassom *et al*., 2008).

We are inclined to explain the increased content of oleate in carriers of the *SU2* mutation and *O2SU2* gene combination precisely by the effect of spatial linkage of the *SU2* gene with the oleate-coding locus of chromosome 6. Among the inbreds analyzed in the experiments, only the inbreds carrying the recessive *SU2* alleles showed an increased content of oleate. In addition, among the carriers of the *SU2* mutation and the *O2SU2* gene combination, there were, although rarely, inbreds with the usual oleate content, and their appearance can be explained by the effect of the crossover distribution of the *SU2* locus and the oleate-coding locus linked to it.

The results obtained showed that within all analyzed groups of inbreds, there was a quantitative variability of grain quality traits. Its main reason should be to recognize the effects of polygenic complexes, which cause an independent effect on these characters (Guttierrez-Rojas *et al*., 2010; Yang *et al*., 2010; Li *et al*., 2018; Leng *et al*., 2019) and can modify phenotypic manifestation of mutant genes *O2* and *SU2,* as well as the effect of their non-allelic interaction.

CONCLUSION

In terms of the complex biochemical characters, the inbreds – carriers of the *O2SU2* gene combination are superior not only to the common type corn inbreds but also to the inbreds - carriers of monogenic *O2* and *SU2* mutations. In comparison with mutants *O2* they were distinguished by an increased content of protein (by 12,3% on average), amylose in starch (by 38,9% on average), starch digestibility (by 24,4% on average), oil content (by 18,4% on average) and oleate content in oil (by 29,9% on average). In comparison with the carriers of *SU2* mutation, they had a higher content of lysine and tryptophan in the total grain protein (on average, by 19,4% & 14,3%, respectively). The main characteristics of grain quality in carriers of a combination of mutant genes *O2SU2* were characterized by quantitative variability, which can modify the effect of non–allelic interaction of mutant genes *O2* and *SU2* The obtained results indicate the effectiveness of using non-allelic interactions between the O2 and SU2 mutant genes to improve the quality of corn grain

ACKNOWLEDGEMENTS

The authors express their deep gratitude to the staff of the NASKO Seed Breeding Station and its director Viktor Muzhilko for the help in organization and conducting field experiments on growing corn inbreds for this study.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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